Comparison of MB/BacT ALERT 3D System with Radiometric BACTEC System and Löwenstein-Jensen Medium for Recovery and Identification of Mycobacteria from Clinical Specimens: a Multicenter Study

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The MB/BacT ALERT 3D System (MB/BacT) (Organon Teknika, Boxtel, The Netherlands) is a fully automated, nonradiometric system with a revised antibiotic supplement kit designed for the recovery of mycobacteria from clinical specimens. In a multicenter study, the recovery rate of acid-fast bacilli (AFB) and the mean time to their detection from clinical specimens was determined by using the MB/BacT system. Data were compared to those assessed by the radiometric BACTEC 460 system (B460) and by culture on Löwenstein-Jensen (L-J) solid medium. A total of 2,859 respiratory and extrapulmonary specimens were processed by the *N*-acetyl-L-cysteine (NALC)-NaOH method using two different concentrations of sodium hydroxide; 1.5% was adopted in study design A (1,766 specimens), and 1.0% was used in study design B (1,093 specimens). The contamination rates for MB/BacT were 4.6% (study design A) and 7.1% (study design B). One hundred seventy-nine mycobacterial isolates were detected by study design A, with 148 *Mycobacterium tuberculosis* complex (MTB) isolates and 31 nontuberculous mycobacteria (NTM) isolates. Overall recovery rates were 78.8% for MB/BacT ($P = 0.0049$), 64.2% for L-J ($P < 0.0001$), and 87.1% for B460, whereas they were 84.5, 70.9, and 91.2%, respectively, for MTB alone. A total of 125 mycobacteria were detected by study design B, with 46 MTB and 79 NTM. Overall recovery rates by the individual systems were 57.6% ($P = 0.0002$), 56.8% ($P = 0.0001$), and 80% for MB/BacT, L-J, and B460, respectively, whereas the rates were 91.3, 78.3, and 97.8% for MTB alone. By study design A, the mean times to detection of smear-positive MTB, smear-negative MTB, and NTM were 11.5, 19.9, and 19.6 days, respectively, with the MB/BacT; 8.3, 16.8, and 16.6 days, respectively, with the B460; and 20.6, 32.1, and 27.8 days, respectively, with L-J medium. By study design B, the mean times were 15.1, 26.7, and 26 days with the MB/BacT; 11.7, 21.3, and 24.8 days with the B460; and 20.4, 28.7, and 28.4 days with L-J medium. Identification was attempted by probing (Accuprobe) MB/BacT-positive bottles within the first working day following instrument positive flag. Results were compared to those obtained in the B460 positive vials by the *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test (study design A) or by the Accuprobe assay (study design B). About 90% of MTB and 100% of NTM could be identified, showing turnaround times closely related to those obtained by combining B460 and the NAP test or the Accuprobe assay. In conclusion, even though recovery rates were shown to be lower than B460, especially for NTM, and contaminants were somewhat higher, MB/BacT represents a valuable alternative to the radiometric system, especially in those laboratories where disposal of radioactive waste is restricted. Finally, when AFB are cultured in nonradiometric liquid media, our data (detection times and bacterial overgrowth rates) suggest that decontamination with 1.5% NaOH may be more suitable than the standard NALC-NaOH.

Although the introduction of amplification techniques in the mycobacteriology laboratory is going to provide faster and more accurate detection of *Mycobacterium tuberculosis* complex (MTB) from respiratory and extrapulmonary specimens, culture still represents a decisive step for diagnosis, treatment, and control of tuberculosis.

A combination of solid and liquid media is currently regarded as the "gold standard" for primary isolation of mycobacteria, and turnaround times not exceeding 21 to 30 days after specimen collection are recommended for MTB identification and drug susceptibility testing (5, 19, 20). Laboratories attempting to meet these requirements face limited choices of culture test systems. Culture on solid media is labor-intensive and it may take several weeks for colonies to become detectable; even then, the process may require further subculture for definitive identification. Manual liquid medium methods are faster and sensitive, but handling of culture vials for visual inspection or reading is cumbersome and time-consuming (11, 13, 14, 22). Limitations of the radiometric system are well known and include manual loading, potential risk of cross contamination related to the invasive reading, lack of computerized data management, and overall the accumulation of radioactive waste (6, 7). Recently, several new, nonradiometric

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culture systems featuring continuous monitoring of growth have been introduced in order to circumvent these drawbacks (12, 23, 24, 25).

Organon Teknika Corp. (Boxtel, The Netherlands) has developed the continuously monitored nonradiometric MB/BacT ALERT 3D system (MB/BacT), which includes a computerized database management system. Carbon dioxide released into the medium by actively growing mycobacteria is detected through a gas-permeable sensor containing a colorimetric indicator embedded at the bottom of culture vials. Color changes are monitored by a reflectometric detection unit contained inside each incubating drawer of the instrument. The purpose of this multicenter study was to evaluate the performance of the MB/BacT system, in comparison with the BACTEC 460 TB system (B460) (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) and Löwenstein-Jensen (L-J) solid medium, for recovery rates and the mean time required to detect and identify mycobacteria from respiratory and extrapulmonary specimens. Moreover, in order to evaluate the impact of decontamination on overgrowth rates and mean detection times, two different procedures were adopted.

(These results were partially presented at the 100th General Meeting of the American Society for Microbiology in Los Angeles, Calif. [C. Piersimoni, C. Scarparo, A. Callegaro, E. Ausili, C. Passerini Tosi, M. Scagnelli, A. Rigon and A. Ruggiero, Abstr. 100th Gen. Meet. Am. Soc. Microbiol. 2000, abstr. U-67, p. 657, 2000]).

MATERIALS AND METHODS

Specimen collection and processing. This multicenter study included 2,859 clinical specimens, both respiratory and extrapulmonary, consecutively received for mycobacterial culture in three Italian microbiology laboratories (site 1, Ancona; site 2, Bergamo; site 3, Vicenza). Blood specimens were excluded from the study. Clinical samples were obtained from 1,804 patients who were almost entirely admitted to hospitals with respiratory symptoms or septicemia. Sites 1 and 2 (study design A) processed 1,766 specimens (864 and 902 specimens, respectively), while site 3 (study design B) tested 1,093 specimens. Investigated specimens included 1,277 sputa, 578 bronchial washings, 552 urine, 259 normally sterile body fluids (pleural, pericardial, synovial, and cerebrospinal fluids and ascites), 70 biopsies, and 69 miscellaneous samples such as pus and gastric aspirate specimens.

In study design A, specimens collected from contaminated sites were liquefied and decontaminated with an equal volume of *N*-acetyl-L-cysteine (NALC) and 3% sodium hydroxide (final concentration, 1.5%) for 15 min at room temperature. In study design B, specimens were liquefied and decontaminated by the standard NALC procedure (10). After decontamination, all specimens were neutralized with phosphate-buffered saline (0.067 M, pH 6.8) and centrifuged at $3,500 \times g$ for 20 min. The pellet was used for smear preparation, resuspended in phosphate-buffered saline to a final volume of 1.5 ml, and inoculated into culture medium. Specimens collected from sterile sites were concentrated by centrifugation without prior decontamination.

Media and culturing methods. The MB/BacT system consists of a bottle containing 10 ml of modified Middlebrook 7H9 broth enriched with casein, bovine serum albumin, and catalase. Shortly before specimen inoculation, bottles were supplemented with 0.5 ml of MB/BacT antibiotic supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin), which was reconstituted with 10 ml of MB reconstituting fluid (Tween 80, glycerol, amaranth, and purified water) according to the manufacturer's instructions. After inoculation, bottles were introduced into the BacT ALERT 3D instrument (Organon Teknika) and incubated at 37°C for 6 weeks. According to the manufacturer's recommendations, mycobacterial strains, which were obtained from the American Type Culture Collection (ATCC) and included *M. tuberculosis* H37Rv ATCC 27294, *M. kansasii* ATCC 12478, *M. avium* ATCC 1576, and *M. fortuitum* ATCC 6841, were tested with each new batch of MB/BacT medium. A few colonies of the test organism growing on L-J subculture slants were emulsified in sterile saline with glass beads. The turbidity of the mycobacterial sus-

TABLE 1. Study design A: recovery rates of mycobacteria by different systems

Mycobacterial species (n)	No. (%) of positive specimens detected by:[a]		
	MB/BacT	B460	L-J medium
MTB (148)	125 (84.5)	135 (91.2)	105 (70.9)
M. tuberculosis (146)	125	133	105
M. bovis (2)	0	2	0
Smear positive (67)	63	65	62
Smear negative (81)	62	70	43
NTM (31)	16 (51.6)	21 (67.7)	10 (47.1)
M. gordonae (14)	5	10	2
M. avium complex (4)	4	3	2
M. chelonae (4)	2	1	3
M. kansasii (3)	3	3	2
M. fortuitum (3)	1	2	0
M. marinum (2)	0	2*	1*
M. xenopi (1)	1	0	0
Total (179)	141 (78.8)[#]	156 (87.1)[#]	115 (64.2)[#]
All clinically significant mycobacteria (162)	135 (83.3)	144 (88.9)	113 (69.7)

[a] #, chi-square test for differences in recovery rates: B460 versus MB/BacT, $P = 0.049$; B460 versus L-J, $P < 0.0001$; MB/BacT versus L-J, $P = 0.003$. *, cultures incubated at 30°C.

pension was adjusted to match that of 0.5 McFarland standard, and 0.1 ml of serial dilutions (10^{-3} and 10^{-4}) of this suspension was inoculated into the MB/BacT bottles.

For mycobacterial culture, each processed sample (0.5 ml) was randomly inoculated into liquid and solid media. B460 12B medium vials were supplemented with 0.1 ml of PANTA antimicrobial supplement. After inoculation, L-J slants (BBL Microbiology Systems, Cockeysville, Md.) were kept for a week with the caps loose, in order to enhance sample evaporation, and later tightened. All media were incubated at 36 ± 1°C for 6 weeks. The radiometric growth index of the 12B vials was recorded by the B460 instrument twice a week for the first 3 weeks and weekly thereafter. A growth index (GI) of >30 was regarded as positive, and smears were made to confirm the presence of mycobacteria. The same procedure was carried out when MB/BacT bottles were flagged positive by the instrument. L-J slants were visually inspected for growth on the slant once a week and smears from suspect colonies were made.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome or by the Ziehl-Neelsen method to detect mycobacteria.

Work-up of discrepant cultures. Whenever growth was detected in only one liquid system, the negative culture of the other system was subcultured onto 7H11 medium at the end of the incubation period. Negative test samples found to be positive on subculture were recorded as false negatives. Similarly, samples flagging positive for three consecutive times without any microscopic or cultural evidence of mycobacterial growth (by subculture onto 7H11 at the end of the incubation period) were considered false positives.

Identification of mycobacteria. Isolates were identified by specific DNA probes (Accuprobe; Gen-Probe Inc., San Diego, Calif.) (15), by the radiometric *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test (Becton Dickinson Diagnostic Systems) (18) and by conventional biochemical and culture tests (7). Some of the isolates were identified by their pattern of mycolic acids by using high performance liquid chromatography (21).

(i) DNA probes. From positive liquid media, 2 ml were drawn and centrifuged for 20 min at $3,500 \times g$ and the pellet was used in the hybridization test. At sites 1 and 3, samples were read in a Leader 50 luminometer (Gen-Probe Inc.), while at site 2 a PAL luminometer (Gen-Probe Inc.) was used. Samples producing signals greater than 30,000 RLUs (Leader 50 luminometer) or 900 PLUs (PAL luminometer) were considered positive (15). Cultures were probed on the first working day following MB/BacT instrument positive flag, or after the GI reached ≥600 for the B460. Starting from the evidence of a very high number of acid-fast bacilli (AFB) in the MB/BacT medium since the first signal, this procedure was adopted in order to shorten identification time as much as possible. Specific probes were chosen on the basis of AFB microscopic appearance in liquid media (8) and pellet pigmentation. If cord formation was seen on Ziehl-Neelsen stain-

TABLE 2. Detection times of mycobacteria in clinical specimens by different systems

Study design	Mycobacterial isolates (n)	Mean detection time (days)					
		MB/BacT		B460		L-J medium	
		Avg	Range	Avg	Range	Avg	Range
A	MTB						
	Smear positive (67)	11.5	5–36.6	8.3	1–28	20.6	9–57
	Smear negative (81)	19.9	7–40	16.8	7–40	32.1	13–57
A	NTM (31)	19.6	2–39	16.6	5–41	27.8	11–42
B	MTB						
	Smear positive (34)	15.1	6–47	11.7	2–34	20.4	11–62
	Smear negative (12)	26.7	10–40	21.3	10–29	28.7	7–53
B	NTM (79)	26.0	3–75	24.8	4–65	28.4	7–63

ing, only the MTB probe was used. Accuprobe-negative cultures suspected to be MTB strains were retested after additional incubation.

(ii) NAP test. The NAP test was performed according to the manufacturer's instructions. A decrease or lack of change in the GI in the presence of NAP over a 3- to 5-day period was considered evidence of MTB.

In study design A, MTB strains detected by the radiometric system were identified by the NAP test, while those grown in the MB/BacT bottles were identified by DNA probes. In study design B, all MTB strains detected by liquid media were identified by DNA probes. Cumulative times, obtained by adding identification times to detection times, were compared.

Statistical analysis. The statistical significance in recovery rates was determined by the chi-square test (Epi Info version 6.03; Centers for Disease Control and Prevention). The statistical differences in the number of days required to recover mycobacteria were determined by Student's t test. P values of ≤ 0.05 were considered significant.

RESULTS

Three-hundred four mycobacterial isolates grew from 2,859 specimens. Of these isolates, 117 (38.5%) were smear positive and 187 (61.5%) were smear negative. The mycobacterial isolates included MTB ($n = 194$), *M. gordonae* ($n = 43$), *M. avium* complex (MAC) ($n = 10$), *M. kansasii* ($n = 7$), *M. chelonae* ($n = 17$), *M. fortuitum* ($n = 9$), *M. marinum* ($n = 2$), *M. xenopi* ($n = 12$), *M. terrae* complex ($n = 3$), and others ($n = 7$). The percentage of all specimens testing positive for any mycobacteria was 10.6%, whereas the MTB isolation rate was 6.8%.

Study design A. Table 1 summarizes the recovery rates for mycobacteria by each culture system adopted in study design A. The MB/BacT and B460 systems detected 78.8 and 87.1%, respectively, while L-J detected 64.2% of all isolates. Statistically significant differences were found not only between the two liquid media and L-J (P, 0.003 and <0.0001 for MB/BacT and B460, respectively) but also between B460 and MB/BacT ($P = 0.049$). While the different liquid media showed no statistically significant differences for the isolation of MTB strains (84.5 and 91.2% for the MB/BacT and B460 systems, respectively), NTM recovery rate was considerably higher by the B460 system (67.7%) than by the MB/BacT system (51.6%). Major differences were observed for *M. gordonae* and *M. marinum*, even though for the latter species the optimal growth temperature cannot be achieved by the 37°C-incubating MB/BacT instrument. Overall detection rates for clinically significant mycobacteria were 83.3% for MB/BacT, 88.9% for B460, and 69.7% for L-J. The difference between B460 and MB/BacT was not significant.

An evaluation of solid-plus-liquid-media combinations revealed a recovery rate of 85.5% mycobacterial isolates for MB/BacT plus L-J (153 strains, with 132 MTB and 21 NTM strains), while B460 plus L-J, detecting a total of 167 strains including 141 MTB and 26 NTM, obtained a slightly higher yield (93.3%). No statistically significant differences were found between media combinations. Table 2 details the time required for each culture system to detect mycobacterial growth. A comparison of the isolation time for MTB strains recovered from smear-positive specimens showed that MB/BacT, B460, and L-J medium detected mycobacteria growth after 11.5, 8.3, and 20.6 days, respectively. For smear-negative MTB-yielding specimens, the isolation times were 19.9, 16.8, and 32.1 days, respectively. The average times for detection of NTM were 19.6 (MB/BacT), 16.6 (B460), and 27.8 days (L-J medium).

(i) Identification. Identification results are shown in Table 3. The NAP test allowed correct identification of 100% of MTB strains detected by the B460 system. Average NAP test time was 3.6 days (range, 3 to 5 days) for smear-positive cultures and 3.5 days (range, 3 to 6 days) for smear-negative ones.

TABLE 3. MTB identification time: MB/BacT plus Accuprobe versus B460 plus NAP test or B460 plus Accuprobe

Study design	MTB isolates	MB/BacT			B460		
		Detection time (days)	Accuprobe cumulative time	% Identified strains	Detection time (days)	Cumulative time[a]	% Identified strains
A	Smear positive	11.5	12.0	94.3	8.3	11.9	100
A	Smear negative	19.9	20.4	84.9	16.8	20.3	100
B	Smear positive	15.1	15.6	90.3	11.7	14.7	100
B	Smear negative	26.7	27.2	81.8	21.3	25.3	100

[a] Cumulative time by NAP test (study design A) or Accuprobe (study design B).

TABLE 4. Study design A: Accuprobe identification from MB/BacT-positive vials

Site no.	AFB isolates (n)	No. tested	No. positive	Avg RLUs/ PLUs	Range RLUs/ PLUs
1	MTB				
	Smear positive (49)	45	42	261,249	31,135–495,026
	Smear negative (49)	40	35	228,373	48,981–377,594
2	MTB				
	Smear positive (18)	8	8	3,661	982–10,987
	Smear negative (32)	13	10	7,019	1,042–12,530
Both sites	NTM (31)	7	7	ND[a]	ND

[a] ND, not done.

Cumulative identification times (detection plus NAP test) were 11.9 and 20.3 days for smear-positive and smear-negative MTB cultures, respectively. Accuprobes performed within the first working day (12 h) following MB/BacT instrument signal allowed correct identification in 94.3% of smear-positive MTB cultures and 84.9% of smear-negative ones. Therefore, for the majority of MTB-yielding cultures, cumulative identification times (detection plus Accuprobe) were 12.0 and 20.4 days, respectively. Although some hybridization tests produced signals slightly above the cutoff, on average the positive values (RLUs/PLUs) could be clearly distinguished from negative ones (Table 4). Of the 11 bottles which did not yield a positive direct DNA probe result, 6 turned out to be positive when tested after incubation at 35°C for 1 week and 5 gave negative results even after a supplementary 2-week incubation period and probing the pellet that was obtained by centrifuging the whole vial content. At visual inspection, these cultures showed a different pattern of growth: instead of the usual abundant, microfloccular growth, the cultures were characterized by a few crumb-like particles which required a 19-gauge needle to be withdrawn. These isolates were identified by conventional tests applied on subculture growth. Seven cultures yielding NTM were also tested by Accuprobe, with the probe selected on the basis of AFB microscopic appearance in MB/BacT medium and pellet pigmentation. All these cultures (*M. gordonae* [*n* = 3], MAC [*n* = 3], and *M. kansasii* [*n* = 1]) were positive on the first attempt.

(ii) Bacterial overgrowth. L-J slants had the highest rate of contamination (7.8%), while MB/BacT and B460 systems showed contamination rates of 4.6 and 3.8%, respectively. The most frequent contaminants in both the liquid media were represented by gram-positive organisms. Despite the introduction of vancomycin in the antimicrobial supplement, 47 MB/BacT cultures were contaminated by staphylococci and 21 cultures were contaminated by other gram-positive bacilli, while B460 had 31 and 18 contaminants, respectively. Gram-positive organisms (67 samples) and *Pseudomonas* spp. (40 samples) were the L-J medium's prevalent contaminants.

Study design B. Table 5 reports the recovery rates for mycobacteria by each culture system adopted in study design B. The MB/BacT and B460 systems detected 57.6 and 80%, respectively, while L-J detected 56.8% of all isolates. Statistically significant differences were observed between the B460 system and either the MB/BacT system or L-J medium (*P*, 0.00022 and 0.00013, respectively). As with study design A, no statistically significant differences were found for MTB isolates (91.3, 97.8, and 78.3% recovery for MB/BacT, B460, and L-J, respectively),

while NTM were detected with a strikingly higher rate by B460 (69.6%) compared with those achieved by MB/BacT and L-J medium (38 and 44.3%). In particular, major differences were observed for *M. gordonae*, *M. xenopi*, and *M. chelonae*. However, when clinically significant mycobacteria species were considered, overall detection rates by MB/BacT and B460 were similar (90.2 and 98.0%, respectively), while L-J detected only 69.7%. The difference between B460 and MB/BacT was not significant. An evaluation of solid-plus-liquid-medium combinations revealed a recovery rate of 78.4% mycobacterial isolates for MB/BacT medium plus L-J (45 MTB and 53 NTM), while the B460 system plus L-J obtained a significantly higher (*P* = 0.002) yield (92.8%), detecting 116 strains including 46 MTB and 70 NTM. The difference between the recovery rates of B460 and the combination of B460 and L-J turned out to be statistically significant (*P* = 0.05).

Table 2 details the time required by each culture system to detect mycobacterial growth. MTB strains were detected from smear-positive specimens after 15.1 and 11.7 days when using the MB/BacT and B460 systems, respectively, and after 20.4 days when using L-J medium. For smear-negative MTB-yielding specimens, the detection times were 26.7, 21.3, and 28.4 days, respectively. NTM were recovered on average after 26 (MB/BacT), 24.8 (B460), and 28.4 (L-J) days.

TABLE 5. Study design B: recovery rates of mycobacteria by different systems

Mycobacterial species (n)	No. (%) of positive specimens detected by:[a]		
	MB/BacT	B460	L-J medium
MTB (46)	42 (91.3)	45 (97.8)	36 (78.3)
M. tuberculosis (46)	42	45	36
Smear positive (34)	31	34	27
Smear negative (12)	11	11	9
NTM (79)	30 (38.0)	55 (69.6)	35 (44.3)
M. gordonae (29)	9	13	15
M. avium complex (6)	6	6	6
M. chelonae (13)	0	13	4
M. kansasii (4)	4	4	2
M. fortuitum (6)	3	3	1
M. terrae complex (3)	1	2	0
M. xenopi (11)	5	11	4
Unclassified (7)	2	3	3
Total (125)	72 (57.6)[#]	100 (80.0)[#]	71 (56.8)[#]
All clinically significant mycobacteria (51)	46 (90.2)	50 (98.0)	38 (74.5)

[a] #, chi-square test for differences in recovery rates: B460 versus MB/BacT, *P* = 0.0002; B460 versus L-J, *P* = 0.0001; MB/BacT versus L-J, *P* = 0.89.

(i) Identification. Identification results are shown in Table 3. Although Accuprobe allowed correct identification of 100% of the MTB strains detected by B460, the strains could be successfully probed an average of 3 days (range, 2 to 4 days) after radiometric detection for smear-positive specimens and 4 days (range, 3 to 5 days) after radiometric detection for smear-negative ones. Cumulative identification times (detection plus Accuprobe) were 14.7 and 25.3 days for smear-positive and smear-negative MTB cultures, respectively.

Accuprobe performed within the first working day (12 h) following MB/BacT instrument signal allowed correct identification in 90.3% of smear-positive cultures (28 out of 31) and 81.8% of smear-negative ones (9 out of 11). Therefore, for the majority of MTB-yielding cultures, cumulative identification times (detection plus Accuprobe) were 15.6 and 27.2 days, respectively. No significant differences in either the average or range of positive values (RLUs) were obtained with Accuprobe assays from MB/BacT or B460 liquid media. Of the five bottles which did not yield a positive direct DNA probe result at the first attempt, all were positive when tested after incubation at 35°C for 1 week.

(ii) Bacterial overgrowth. The nonmycobacterial overgrowth rate in decontaminated specimens was somewhat higher for the MB/BacT system (7.1%) than for the B460 system and L-J medium (4.6 and 3.8%, respectively). The most frequent contaminants in either liquid medium were represented by gram-positive organisms. Fifty MB/BacT cultures were contaminated by staphylococci and 18 cultures were contaminated by other gram-positive bacilli, while B460 had 17 and 26 contaminants, respectively. Gram-positive organisms (24 samples) and *Pseudomonas* spp. (14 samples) were prevalent in L-J medium.

False-positive and false-negative rates. No false-positive cultures were signaled by the MB/BacT instrument, while two false-negative cultures were detected. In both cases, subculture on Middlebrook 7H11 medium yielded MTB isolates which were fully susceptible to antituberculous drugs.

DISCUSSION

Despite considerable improvement of commercially available assays and their advantage in shortened turnaround times for diagnosis, nucleic acid amplification techniques are not expected to supplant culture for the definitive diagnosis of clinically significant mycobacterial infections. Top priorities for diagnostic laboratories dealing with large specimen loads such as sensitive mycobacterial growth detection and automation of the cultivation process, strongly stimulated technical developments by almost all companies working in the field of mycobacteriology. Many new manual or automated systems have been developed. The MB/BacT system is a fully automated, nonradiometric system for the culture of mycobacteria which was approved by the Food and Drug Administration in 1996.

In the present study, the MB/BacT Alert 3D with a revised reconstitution fluid-antibiotic supplement was compared with the radiometric B460 system and L-J medium. We have evaluated the recovery rates and time to detection of mycobacteria from approximately 2,900 specimens by each system alone and also by system combinations using two different decontamination procedures.

As no individual medium correctly detected all mycobacte-ria, it is emphasized that in order to get optimal recovery of mycobacteria from clinical specimens, a combination of liquid and solid media is essential. In this context, discrepancies between liquid media were more pronounced with smear-negative MTB and with some NTM species. This held true especially for *M. chelonae*, *M. gordonae*, *M. xenopi*, and *M. marinum*. Unfortunately, the MB/BacT instrument incubates culture vials at one temperature only, 37°C, which is a temperature that supports the growth of most clinically significant mycobacteria but is not optimal for *M. chelonae*, *M. haemophilum*, *M. marinum*, and *M. ulcerans*. Moreover, should MB/BacT vials be incubated out of the 3D system, they can be neither read visually (because CO_2 production is low and sensor change of color cannot be detected by visual inspection) nor inserted into the instrument for only the reading. In this case, the manufacturer suggests testing MB/BacT vials for visual evidence of growth followed by subculture on solid media. In our study, we limited the incubation period to 6 weeks (as recommended by the manufacturer) and did not evaluate the impact of a prolonged incubation time on MB/BacT recovery rates (especially NTM). Comparing the individual performance of each method, both liquid media were superior to the conventional L-J medium, a result which is now well established for the majority of liquid media (6).

In this evaluation the time until detection of mycobacteria was on average 3.2 days longer for MB/BacT than for B460. This trend was confirmed for all categories of specimens, with mean differences of 3.3 days (range, 3.2 to 3.4 days) for smear-positive MTB, 4.2 days (range, 3.1 to 5.4 days) for smear-negative MTB, and 2.1 days (range, 1.2 to 3.0 days) for NTM. Pair-wise comparisons of mean mycobacteria detection times were statistically significant for B460 versus MB/BacT medium ($t = 5.08$; $P < 0.0001$), as well as for MB/BacT medium versus L-J medium ($t = 8.07$; $P < 0.0001$). Our data are in agreement with previous studies which reported MTB detection times ranging from 11.8 to 21 days for MB/BacT and from 8 to 18 days for the B460 system (1, 3, 4, 9, 16, 17).

The bacterial overgrowth rate was higher in the MB/BacT bottles than in B460 12B vials. It was more pronounced in study design B (7.1% versus 4.6%), in which a milder decontamination method was employed, than in study design A (4.6% versus 3.8%). This relationship has been described in many comparison studies (1, 3, 9, 16, 17), and even after the antibiotic supplement was revised by adding vancomycin in an attempt to reduce gram-positive overgrowth, the contamination rate was still higher than that of B460, and gram-positive organisms were the prevalent contaminants. In study design A, 7.8% of the L-J cultures were contaminated with microorganisms other than mycobacteria, compared to 3.8% of cultures in study design B. *Pseudomonas* spp. accounted for the higher number of contaminated L-J cultuers found in study design A.

Adopting two different decontamination procedures, we expected to find fewer contaminants, coupled with longer detection times, in study design A (1.5% NaOH [final concentration]) and the opposite in study design B (1.0% NaOH [final concentration]). While the overgrowth rates met our expectations, we surprisingly found similar or shorter detection times with both liquid media when using a harsher decontamination procedure. In the category of smear-positive MTB-yielding samples, average detection times were apparently different de-

pending on the uneven distribution of (+++), (++), and (+) smear-positive samples within each study design. In fact, average detection times inside each subset of smear-positive samples were similar. For smear-negative MTB-yielding samples, even considering the different number of specimens within each study design and the unpredictable load of viable mycobacteria, the difference in detection times was considerable. For NTM, a considerable number of recovered strains, including many slow growing species like *M. xenopi*, and the low rate of MAC isolates (an appreciable effect of the recently introduced antiretroviral therapies) may justify the longer detection time found in study design B. Moreover, the higher yield may depend not only on the different population referring to the hospital (the majority were AIDS or human immunodeficiency virus-positive patients) but also on the milder decontamination procedure.

Identification of mycobacteria to species level was attempted directly from positive MB/BacT bottles by using the Accuprobe assay. This procedure was compared with identification from positive B460 vials by the NAP test in study design A and by the Accuprobe assay in study design B. While 12B vials cannot be probed with reliable results until the GI exceeds 500 to 600, thereby requiring additional incubation days following initial detection, and the NAP test requires 3 to 4 days to identify MTB strains, MB/BacT bottles can be successfully probed on the first working day as the bottle is flagged positive by the instrument. We found that more than 90% of smear-positive MTB strains (94.3% in study design A and 90.3% in study design B), more than 80% of smear-negative MTB strains (84.93% in study design A and 81.8% in study design B), and 100% of NTM strains could be probed within 12 h following instrument positive signal, thus offsetting the initial advantage of a faster detection by the B460 system. The specific probe was chosen according to the cord-like microscopic appearance in broth, pigmentation of the culture pellet, and local prevalence of mycobacterial species. Differently from Badak et al. and Benjamin et al. (2, 3), we concentrated MB/BacT cultures by centrifugation before probing them. On the basis of our data, we believe that this step is essential, especially for those MTB cultures showing defective growth, whose cell mass may be scant even after centrifugation.

Finally, each liquid medium can be evaluated for the following advantages. (i) The B460 provides faster (on average 3.2 days earlier) and more sensitive detection of positive cultures, especially for NTM; (ii) a more accurate recovery of MTB isolates, even though not statistically significant compared to the MB/BacT system, may be relevant in labs where a low TB prevalence occurs; (iii) overgrowth with nonmycobacterial organisms is less frequent; and (iv) individual vials can be incubated at different temperatures. By contrast, the MB/BacT system is marked by the following: (i) it is a closed system with no cross-contamination risk, (ii) the majority of mycobacterial isolates can be promptly identified by the Accuprobe assay, and (iii) it is a fully automated walk-away system which does not generate radioactive waste.

We conclude that the MB/BacT system is an automated, rapid, and less labor-intensive mycobacterial culturing system which may be considered a valuable alternative to the semiautomated radiometric system, especially in those laboratories where disposal of radioactive waste is restricted. When AFB are cultured employing rich liquid media like MB/BacT or other nonradiometric ones, decontamination with 1.5% NaOH may be more suitable than the standard NALC-NaOH.

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